



LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

Enhancement of T1 and T2 relaxation by paramagnetic silica-coated nanocrystals

Daniele Gerion, Julie Herberg, Erica Gjersing, Erick Ramon, Robert Maxwell, Joe W. Gray, Thomas F. Budinger, Fanqing Frank Chen

August 29, 2006

Journal of Physical Chemistry C

This document was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor the University of California nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or the University of California, and shall not be used for advertising or product endorsement purposes.

Enhancement of T_1 and T_2 relaxation by paramagnetic silica-coated nanocrystals

Daniele Gerion,^{1,*} Julie Herberg,¹ Erica Gjersing,¹ Eric Ramon¹
Robert Maxwell¹, Joe W. Gray², Thomas F. Budinger², Fanqing Frank Chen²

¹*Lawrence Livermore National Laboratory, Livermore, CA, 94551;*

²*Lawrence Berkeley National Laboratory, Berkeley, CA 94720*

Abstract

We present the first comprehensive investigation on water-soluble nanoparticles embedded into a paramagnetic shell and their properties as an MRI contrast agent. The nanoprobes are constructed with an inorganic core embedded into an ultra-thin silica shell covalently linked to chelated Gd^{3+} paramagnetic ions that act as an MRI contrast agent. The chelator contains the molecule DOTA and the inorganic core contains a fluorescent CdSe/ZnS qdots in Au nanoparticles. Optical properties of the cores (fluorescence emission or plasmon position) are not affected by the neither the silica shell nor the presence of the chelated paramagnetic ions. The resulting complex is a MRI/ fluorescence probe with a diameter of 8 to 15 nm. This probe is highly soluble in high ionic strength buffers at pH ranging from ~4 to 11. In MRI experiments at clinical field strengths of 60 MHz, the QDs probes posses spin-lattice (T_1) and a spin-spin (T_2) relaxivities of $1018.6 \pm 19.4 \text{ mM}^{-1} \text{ s}^{-1}$ and $2438.1 \pm 46.3 \text{ mM}^{-1} \text{ s}^{-1}$ respectively for probes having ~ 8 nm. This increase in relaxivity has been correlated to the number of paramagnetic ions covalently linked to the silica shell, ranging from approximately 45 to over 320. We found that each bound chelated paramagnetic species contributes by over $23 \text{ mM}^{-1} \text{ s}^{-1}$ to the total T_1 and by over $54 \text{ mM}^{-1} \text{ s}^{-1}$ to the total T_2 relaxivity respectively. The contrast power is modulated by the number of paramagnetic moieties linked to the silica shell and is only limited by the number of chelated paramagnetic species that can be packed on the surface. So far, the

sensitivity of our probes is in the 100 nM range for 8-10 nm particles and reaches 10 nM for particles with approximately 15-18 nm in diameter. The sensitivities values in solutions are equivalent of those obtained with small superparamagnetic iron oxide nanoparticles of 7 nm diameter clustered into a 100 nm polymeric shell. A thin paramagnetic silica shell as interface with the bioworld presents several advantages over polymeric coating or dendrimers in terms of *in vivo* biocompatibility and ease of functionalization with targeting biomolecules. Theoretically, these relaxivity values are high enough to be detected by MRI of a single cell labeled with 10^5 probes. We briefly discuss the importance of probes coated with a paramagnetic silica shell for the detection and treatment of diseases *in vivo*.

* to whom correspondence should be addressed: danielegerion@yahoo.com

Recent years have witnessed a new age in fluorescence imaging driven by remarkable advances in detection technologies. The ability to discern fluorescent markers at the single molecule level is important for molecular imaging, in particular for monitoring signaling pathways in live cells or for the study of cellular metabolism.¹ Qdots represent a new form of fluorescent agents that possess many of the properties added to perform these studies.² They have the size of a protein and can be programmed to acquire biological functions, they are well tolerated by live cells, they afford multiplexed detection due to their tunable emission, they are resistant to photobleaching, and they can be tracked at the single molecule level over extended periods of time.^{3, 4} Thus, by using biologically engineered QDs, there is great potential for learning more about the molecular basis of certain diseases, such as cancer, and finding a therapeutic treatment.^{5, 6}

For this noble cause, a big leap forward has to be taken to correlate the deficiency of a cell *in vitro* to a disease *in vivo*. Cells reside in highly structured 3D environments. They are sensitive to the extracellular matrix and to the presence of neighboring cells with which they make mechanical or biochemical contact. Although most diseases originate from the malfunction of a single cell, whether the disease spreads ultimately depends on whether the cell and its daughters can survive and proliferate by beating the feedback control mechanisms in a complex 3D multicellular organism. It is therefore important to know not only what happens within a single cell but also how cells communicate and socialize and how they respond to stimuli *in vivo*.

In this regard, the use of bioengineered QDs is problematic because fluorescence has one notorious shortcoming: light has a penetration depth of less than a few millimeters in the best case. As a result, fluorescence is a powerful tool mainly limited to *in vitro* imaging. *In vivo* medical applications, such as detection of tumors or metastases, or the tracking of stem cells after cell therapy treatment require a different set of non-invasive imaging probes and techniques.⁷⁻⁹

Magnetic resonance imaging is a method of choice for *in vivo* visualization because of its infinite penetration depth and its anatomic resolution. MRI has the ability to map the relaxation processes of water protons in the sample, referred as T_1 and T_2 relaxation times. One of the powers of MRI is its ability to extract image contrast, or a difference in image intensity between tissues, on the basis of variations in the local proton environment. Unfortunately, intrinsic differences between tissues are often too small to provide distinguishable relaxation times for protons. This is why exogenous contrast agents are often used, most notably in the form of small amounts of paramagnetic impurities, such as chelated Gd^{3+} .¹⁰ They accelerate the T_1 and T_2 relaxation processes of water protons in their surrounding. However, contrast between tissues is often too weak for a firm diagnosis. One way to increase contrast is to use an agent that is specifically targets one type of tissue¹¹ Another option is the development of a contrast agent with enhanced sensitivity¹²

The performance of a contrast agent in solution is measured by its relaxivity, defined as $1/T_i \sim r_i * [C]$, $i=1,2$, where r_i is the relaxivity and $[C]$ the concentration of the contrast agent. The higher its relaxivity, the more sensitive the contrast agent. T_1 -contrast agents are agents that affect mostly the longitudinal relaxation time. They are usually

made of chelated lanthanide ions and reach relaxivities of $5\text{-}50\text{ mM}^{-1}\text{ s}^{-1}$.¹³ Higher relaxivities are obtained with T_2 -contrast agents, i.e. agents that affect mainly the transversal relaxation time, which is the most prominent of which are iron oxide nanoparticles. These particles, used in clinical trials, have sizes around 50-200 nm in diameter. At this size range, they often exhibit a hysteresis curve upon application of a reversal magnetic field, a sign that the magnetic moment is tightly bound to the crystal structure. Superparamagnetic behavior (i.e. no hysteresis upon a switching field) is preferred for MRI applications. The SP regime is the realm of smaller magnetic nanoparticles with core sizes below 20 nm, often labeled SPIO (Small Superparamagnetic Iron Oxide).¹⁴ Decreasing the size of the SPIO nanoparticles also leads to a decrease in their total magnetic moment and reduces their contrast power in MRI. In this work, we present and characterize a probe construct that reaches T_1 and T_2 relaxivities of up to approximately $13'000$ and $\sim 15'000\text{ mM}^{-1}\text{ s}^{-1}$ at clinical fields while its size is only 15-20 nm. Smaller constructs of size in the range of 8-10 nm exhibit relaxivities of approximately 1000 and $2000\text{ mM}^{-1}\text{ s}^{-1}$.

The construct consists in an inorganic nanoparticle core (fluorescent CdSe/ZnS or Au) embedded into a thin paramagnetic silica shell. These silica-coated scaffolds are about 10 nm in size and are used to covalently anchor multiple GdDOTA molecules. We present evidence that a multi-component mechanism contributes to these exceedingly high relaxivity values. The mechanism involves a large number of GdDOTA moieties, the slowing of tumbling rate of GdDOTA and the hydrophilicity of the silica surface. In fact, the number of GdDOTA moieties linked to the silica shell can be tuned from 20 up to 320, and each unit contributes additively to the total relaxivity. Moreover, we observe

that the T_1 and T_2 relaxivities per GdDOTA unit (or ion relaxivity) is increased by a factor of 5 and 10 respectively when GdDOTA is bound to the silica shell compared to its mobile form in solution, and by a factor of 2 and 3 respectively compared to the case when GdDOTA is linked to nanoparticles through a flexible, weakly hydrophilic phospholipids layer.

The design of our probe can be easily extended to cover PET imaging, and we do not foresee any difficulty to enhance the capability of the silica-coated probes by adding a targeting mechanism. We will discuss a set of advantages provided by silica over polymeric coatings that have surfaced recently in *in vitro* studies that may pave the way to *in vivo* imaging.

2 Experimental Details

2.1 Synthesis and characterization of silanized CdSe/ZnS QDs and Au nanocrystals coated with Gd-DOTA

In both cases, we use a three-steps process: first we grow a PEG-ylated silica shell containing thiol groups around the inorganic cores (semiconductor or metallic); second we synthesize paramagnetic agents made of an amine-terminated DOTA molecules that chelates Gd^{3+} ions; third we covalently crosslink the chelated paramagnetic compound to silanized particles using a bifunctional cross-linker.

Silanization of QDs: The synthesis and silanization of CdSe/ZnS QDs, or simply $SiO_2@QD$, follow published procedures.^{15, 16} Briefly, after a priming step replacing

TOPO surfactants on the QD surface with MercaptoPropyltrimethoxySilane (MPS), polymerization of siloxane is performed in methanol under slightly basic conditions. In a second step, addition of fresh MPS and PEG-propyltrimethoxysilane permits the introduction of functional (SH) and stabilizing (PEG) groups respectively into the forming SiO₂ shell. Silane polymerization is quenched with trimethylchlorosilane that converts reactive silanol groups into methyl groups. This allows controlling and limiting the size of the silica shell to a thickness of a few nanometers. After extensive dialysis against fresh methanol and subsequently against 10 mM phosphate buffer, pH approximately 7-7.5, silanized QD solutions are concentrated using centricon 100 down to optical densities greater than 30-70 and purified further by low pressure chromatography using a 20 cm long, 1cm ID column filled with sephadex G200 or sephadex G100. Silanized qdots elute in a rather large band. Typically, we load ~ 500-700 µl of solution and collect approximately 5 µl of solution. Solutions of silanized Qdots are stored at room temperature at optical densities in the range of 3-6.

Silanization of Au nanoparticles: A similar approach is used to silanize citrate-stabilized Au nanoparticles of 5 nm and 10 nm purchased at BBI International. However, in the case of colloidal Au, silanization is performed in an aqueous environment because the particles would not disperse in methanol. Since citrate-coated Au nanoparticles aggregate easily even in low ionic strength buffers, the Au colloids are first stabilized with a phosphine surfactant, as we have reported previously^{17, 18}. Phosphine-stabilized Au colloids are precipitated with ethanol and resuspended into a solution of 1:1000 v:v MPS in water to exchange the capping ligands to a thiolated siloxane. After this priming step, an approach similar to the QDs case is taken, with the growth of a shell using MPS

and PEG-silane, quenching of the shell growth using trimethylchlorosilane. All these steps are however performed in water. After the procedure is completed, silanized Au colloids are purified using centrifugation. Silica-coated Au nanocrystals can be concentrated with a centricon 100 device to dryness. Upon addition of buffer, the particles resuspend spontaneously by a gentle shake. Such purification is performed several times. Despite these multiple washing steps and large concentrations (optical densities in the range >100), the plasmon peak of silanized Au colloids measured by UV-Vis does not shift compared to the original diluted samples for both 5 and 10 nm colloids. We take it as a sign that silanization did not induce aggregation of the particles.

The size estimates of the silanized particles are based on previous AFM investigations because the thin silica shell is hardly visible under a TEM. Based on those studies, our estimate is that the silica shell is about 2-3 nm thick and adds 4-6 nm to the particle diameter. In this study, we focus on ~ 5 nm naked CdSe/ZnS, and 5 nm and 10 nm citrate-stabilized Au colloids. After silanization, the respective sizes are ~ 10 nm for silanized QDs, ~ 10 nm for 5-nm Au, and $\sim 16-18$ nm for 10 nm Au.

Gd³⁺ chelation with a DOTA moiety: The synthesis of GdDOTA, *i.e.* one Gd³⁺ ion chelated by DOTA, is performed according to the procedure, adapted from previous reports.^{19, 20} We dissolve p-NH₂-Bn-DOTA, *i.e.* 2-(4-Aminobenzyl)-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid, (Macrocyclics Inc., hereafter called simply DOTA) in water and add an aqueous solution of GdCl₃ (Sigma-Alrich), so as to have a 1: .98 molar mixture of DOTA:Gd³⁺ and ~ 0.2 M concentration in DOTA. The solution is heated a few seconds to 80°C to favor complexation of Gd³⁺ with the tetraazacyclododecane ring. The pH of the solution drops below 1. We bring the pH to

3.5-4 by adding aliquots of 7M NaOH and heat the solution back to 80°C for a few minutes. At this early stage, heating produces acidification of the solution. Therefore, we repeated heating-adjusting the pH to 3.5-4 with sodium hydroxide several times (up to 7 times) until the heating step does not produce a drop in pH below 3.5 is performed. At that stage, the solution is kept at 80°C for 3 hrs. Completion of the Gd³⁺ chelation is confirmed by a colorimetric assay using Arsenazo dye (Sigma-Alrich). This colorimetric dye reacts to the presence of unbound Gd³⁺. The dye natural color is purple, but upon complexion with Gd³⁺, its color turns to blue. After 3 hrs, the GdDOTA solution is slightly yellowish and has a concentration of approximately 150 mM in GdDOTA, deduced from the initial amount of DOTA, GdCl₃ and NaOH used. The stability of the GdDOTA has been studied only summarily using the colorimetric Arsenazo test. No Gd³⁺ release from the DOTA ring was observed over a period of several weeks.

Linking GdDOTA to silanized nanoparticles: Freshly prepared paramagnetic GdDOTA is covalently linked to silanized nanoparticles to form GdDOTA-SiO₂@Particle. First the amino group on the GdDOTA unit is converted into a maleimide group using sulfo-SMCC and classic bioconjugation conditions (pH ~ 6-6.5, SMCC:DOTA ~ 3:1).²¹ After one hour reaction, the maleimide-activated GdDOTA is directly reacted to silanized particles. The reaction is kept running for ~24 hrs at room temperature. Removal of unbound GdDOTA is performed by a 48 hrs dialysis in a 50K MWCO membrane (SpectraPor 6) against a bath consisting in 10 mM phosphate buffer, pH ~ 7. We exchange the buffer bath at least 4 times during the dialysis period. After dialysis, the sample is further purified by 4 to 5 runs of centricon 100. For each run, 2 ml of silanized particles are condensed down to <100 µl and ~ 1.9 ml of fresh buffer is added. After

these extensive purification steps, we estimate that the concentration of unbound GdDOTA is in the fM-pM range, far too small to provide any signal in MRI and far smaller than a few μM s, the typical concentration of silanized nanoparticles.

Determination of the concentration of the samples: The concentrations of our solutions are given in terms of silanized nanoparticle concentration and not in terms of Gd^{3+} present in solution. This seems to be a wise choice, if one considers that all Gd^{3+} are concentrated onto the nanoparticle surface and the solution is not homogenous in Gd^{3+} . [at 1 mM concentration, the average distance between 2 nanoparticles is ~ 100 nm, so Gd^{3+} is highly localized].

We measure the UV-Vis spectrum and deduce the concentration of the solution from the optical density at the exciton (for semiconductors – QDs) or plasmon (for Au colloids) peak using known extinction coefficients and the following equation: $C = \text{OD} / (\epsilon \cdot \delta)$, where OD is the optical density or amplitude of absorption at the exciton/plasmon peak, δ is the cuvette length (usually 1 cm or 2 mm) and ϵ is the extinction coefficient. The extinction coefficients are deduced from the literature (QDs) or given from the manufacturer (Au). We use the following numbers: QD exciton peak at ~ 610 nm, fluorescence emission at ~ 630 nm, fwhm ~ 38 nm, extinction coefficient used $620'000 \text{ M}^{-1}\text{cm}^{-1}$ following published reports.²² For 5 nm and 10 nm Au colloid, both plasmon peaks are at 524 nm and we use $\epsilon = 1,2 \times 10^7 \text{ M}^{-1}\text{cm}^{-1}$ for 5 nm Au and $\epsilon = 1.06 \times 10^8 \text{ M}^{-1}\text{cm}^{-1}$ for 10 nm Au respectively. These latter numbers are computed from the concentrations given by the manufacturer and the OD of citrate-stabilized Au colloids measured directly of the bottle.

Determination of the number of Gd per silanized nanoparticle: After extensive purification from unbound GdDOTA, these samples are chemically analyzed by ICP-MS by measuring the total amount of Gd and Cd or Au ions. By assuming bulk parameters of the CdSe or Au lattice and the size of the nanoparticles (using tabulated values linking the size of the QDs to its optical properties, or the claimed size for Au nanocrystals), we deduce the number of Gd per silanized nanoparticle. The same samples were previously analyzed by MRI. The number of GdDOTA per GdDOTA-SiO₂@Particle varies from ~3 to >250 and depends on the size of the initial nanoparticles and the conditions used during bioconjugation.

2.2 20 MHz and 60 MHz NMR Minispec Parameters

T_1 and T_2 relaxation time measurements were performed on a Bruker Minispecs operating at 20MHz and 60MHz. An inversion recovery pulse sequence was employed for T_1 relaxation time measurements using a mono exponential fit to the recovery curve. For each experiment 4 scans were collected with a recycle delay of 15 seconds. To obtain the recovery curve, 50 evenly spaced points were collected with the first point acquired at 5 ms. The last time point was collected at 4000 ms for short T_1 samples and 10,000 ms for samples with long T_1 so that each sample was allowed to fully recover. The receiver gain for each sample was set so that the signal amplitude was approximately 60%.

T_2 times were calculated from a mono exponential fit to a spin echo decay curve using a CPMG pulse sequence. Eight scans were acquired for each experiment with an

echo time of 1ms, pulse attenuation of 6dB, and recycle delay of 3 seconds. The number of echo times was varied between 100 points, for short T_2 s, and 3500 points, for long T_2 s, in order to acquire the full decay curve for each sample. The receiver gain for each sample was set to the same value that was used in the T_1 experiment.

2.3 Bruker 2.5mm MicroImaging MRI system parameters

All MRI experiments were performed on a Bruker Avance 400 MHz spectrometer equipped with a high-resolution Micro5 microimaging system with a 25 mm rf coil. To obtain spin-lattice relaxation (T_1) values, a Fast Imaging with Steady State Precession (FISP) with Inversion Recovery (IR) Sequence was used. The MRI parameters for FISP include echo time of 1.5 ms, repetition rate of 3.0ms, 8 averages, number of segments of 32, field-of-view of 3x3 cm, resolution of 234x234, microns/pixel, flip angle (alpha) of 60°, and inversion delay (T_1) of 235.5 μ sec. To obtain spin-spin relaxation (T_2) times a Multi Slice Multi Echo (MSME) Sequence was employed. The MRI parameter for MSME including number of echoes of 32, time of echo is 13.8 ms, repetition rate of 10,000 ms, field-of-view of 3x3 cm, resolution of 117x117 microns/pixel, number of averages = 1, and slice thickness = 1 mm.

3. Results

To determine if nanoparticles embedded into a silica shell and linked to chelated paramagnetic ions are sensitive contrast agents for MRI, we first present the case of paramagnetic silanized semiconductor QDs samples, with QD core size of ~ 5 nm.

Subsequently, we will show that paramagnetic silanized metallic Au nanoparticles exhibit similar results. Comparison of MRI data obtained for GdDOTA-SiO₂@P, where P represents nanoparticles of different nature and sizes will shed light on the nature of the mechanism of contrast enhancement.

First images were collected of 4 μ M GdDOTA-SiO₂@QD and compared to control solutions. Control samples include: a) 4 μ M SiO₂@QD; b) 4 μ M DOTA-SiO₂@QD lacking the activation with Gd³⁺; c) 10 mM phosphate buffer, pH \sim 7, the common buffer for these solutions. In the same set of experiments, unbound GdDOTA at concentrations ranging from 390 μ M to 7350 μ M is also profiled. This latter is a clinically approved contrast agent, better known under the trademark DOTAREM[®] and T₁ and T₂ measurements produced similar results to previously reported data²³. Simultaneous data acquisition permits a direct comparison of the performance of current contrast agents with our paramagnetic silica-coated nanoprobe.

Figure 1a shows the spin-lattice relaxation MRI images (T₁ weighted images). Although qualitative differences in the MRI images clearly establish that only paramagnetic GdDOTA-SiO₂@QD provide a marked contrast, a clearer picture emerges from quantitative evaluation of the longitudinal relaxation times T₁, by plotting M/M₀ versus log(τ (ms)), i.e. the residual magnetization as a function of the recovery time. T₁ are measured by fitting M(τ)/M₀ to an single exponential growth curve: (M(τ)/M₀)= (1-exp(τ /T₁)). T₁ recovery curves are not shown; however the values are summarized in Table 1. The GdDOTA-SiO₂@QD sample with a QD concentration of 4 μ M has a T₁ of 186ms, significantly lower than the longitudinal relaxation times of control QD solutions

where T_1 was about 600 ms, or from buffer solutions with $T_1 \sim 420$ ms. The presence of chelated Gd^{3+} linked to the silica shell produces a 3-fold decrease in T_1 .

A similar approach is used to measure the transversal relaxation time T_2 of the same samples. Spin-spin relaxation MRI images (T_2 weighted images) were taken using a Multi Slice Multi Echo (MSME) sequence and are shown in Fig.2. Transversal relaxation times were determined by a plot of $\log (M/M_0)$ versus τ (ms). The T_2 times were computing by fitting $\log(M/M_0)$ to a linear decay curve of $\log (M/M_0) = \tau/T_2$. The T_2 recovery curves are not shown; however the values are summarized in Table 1. The transversal relaxation time of 4 μ M GdDOTA-SiO₂@QD is ~ 77 ms, much shorter than the T_2 of all other control samples where T_2 is in the 345 ms to 425 ms range. In this case, the presence of chelated Gd^{3+} linked to the silica surface produces a 4-fold decrease in T_2 compared to control solutions. As it is clear from Fig. 1, GdDOTA-SiO₂@QD solutions have a stronger influence on the transversal relaxation time than on the longitudinal one.

It is worth noticing that only paramagnetic GdDOTA-SiO₂@QD provide a contrast in MR Images. All solutions lacking the paramagnetic load exhibit MRI images and relaxation times that barely depart from the buffer environment. Moreover, T_1 and T_2 values of 4 μ M GdDOTA-SiO₂@QD solutions are similar to relaxation values measured for unbound GdDOTA with a Gd^{3+} concentration of approximately 800-2500 μ M.²³ It may be tempting to conclude that there are about 200-600 GdDOTA per SiO₂@QD, however such extrapolation will prove to be incorrect as we will discuss later. Similar qualitative results were observed for GdDOTA-SiO₂@Au, with nanocrystal cores of 5 and 10 nm diameter. MRI measurements presented so far indicate that GdDOTA-

SiO₂@QD with a nanoparticle concentration in the μM range has the same contrast power than GdDOTA with Gd³⁺ concentration in mM range.

To quantify this increase in sensitivity, MRI measurements were taken at various concentrations of contrast agent, and relaxivities were computed. GdDOTA-SiO₂@QD with nanoparticle concentration ranging from 0.125 μM to 4 μM and unbound GdDOTA with Gd³⁺ concentration of 390 μM to 7150 μM were investigated simultaneously. The T₁ and T₂ maps are shown in Figure 3a. In the lower panels, we show the linear behaviors of the inverse relaxation times versus the contrast agent concentrations, $1/T_i \sim r_i \cdot C$, $i=1,2$. The slopes r_i represent the relaxivity of GdDOTA-SiO₂@QD (GdDOTA, values in brackets) and correspond to $r_1=807.9 \pm 15.4$ (3.66 ± 0.04) $\text{mM}^{-1}\text{s}^{-1}$ and $r_2=3003.5 \pm 57.1$ (3.8 ± 0.2) $\text{mM}^{-1}\text{s}^{-1}$ at 400 Mhz.

So far, we have focused on the capability of GdDOTA-SiO₂@QD to deliver a contrast signal using a 400 MHz imaging system. However, most clinical scanners operate at 60 MHz. To investigate the behavior of our probes at 20 MHz and 60 MHz, we used a Bruker NMR Minispec. The instrument does not afford imaging but allows the measurement of T₁ and T₂ from which relaxivities are deduced as described above. The longitudinal relaxivity r_1 at 20 MHz and 60 MHz was determined to be $r_1=1932.0 \pm 36.7$ $\text{mM}^{-1}\text{s}^{-1}$ and $r_1=1018.6 \pm 19.4$ $\text{mM}^{-1}\text{s}^{-1}$ respectively. The transversal relaxivity r_2 for 20 MHz and 60 MHz was $r_2=2483.6 \pm 47.2$ $\text{mM}^{-1}\text{s}^{-1}$ and $r_2=2438.1 \pm 46.3$ $\text{mM}^{-1}\text{s}^{-1}$. Relaxivities are summarized in Table 2 and in Fig. 3c. Although we only have three data points, they seem to qualitatively follow the Nuclear Magnetic Relaxation Dispersion (NMRD) profile observed for unbound GdDOTA.^{24, 25} In particular, r_1 is strongly field-dependent. It decreases from ~ 2000 $\text{mM}^{-1}\text{s}^{-1}$ at 20 MHz to ~ 800 $\text{mM}^{-1}\text{s}^{-1}$ at 400 MHz. r_2 ,

as expected, exhibits a very slight increase with increasing fields. Notice how, at all frequencies, the transversal relaxivity of GdDOTA-SiO₂@QD is larger than the longitudinal one. Therefore, GdDOTA-SiO₂@QD appears to be a T₂ contrast agent, even though unbound GdDOTA affects mainly the longitudinal relaxation time.

At the three field strengths investigated here, the relaxivities of GdDOTA-SiO₂@QD reach values over 800-3000 mM⁻¹s⁻¹, while the relaxivities of unbound GdDOTA ceils 3-12 mM⁻¹s⁻¹. We determined by ICP-MS that there are about approximately 45 chelated Gd ions per SiO₂@QD in the samples presented above. Consequently, at 60 MHz for instance, every GdDOTA of the GdDOTA-SiO₂@QD probe contributes for about 22.6 ± 0.4 mM⁻¹s⁻¹ to r₁ and 54.2 ± 0.3 mM⁻¹s⁻¹ to r₂. This represents a six-fold increase for r₁ and twelve-fold increase for r₂ compared to the value found for unbound GdDOTA at the same field strength.

To explore the cause of the increase in ion relaxivity, we initially speculated that the permanent electric dipole of QDs²⁶ may affect the local environment that chelated Gd experience through a dipole coupling. This ultimately perturbs the dynamics of water molecules in their neighborhoods. To test our assumption, QD nanoparticles were replaced with dipole-free cores such as gold. Thus, we developed the silanization of colloidal gold nanoparticles with 5 nm and 10 nm diameter. The smaller Au nanoparticles have the same size than CdSe/ZnS QD cores. For these samples at 20 MHz, we measured r₁ = 2165.8 ± 41.2 mM⁻¹ s⁻¹ and r₂ = 2709.9 ± 51.5 mM⁻¹ s⁻¹ for silanized 5 nm Au colloids, and r₁ = 13510 ± 250 mM⁻¹ s⁻¹ and r₂ = 15815 ± 300 mM⁻¹ s⁻¹ for silanized 10-nm Au. The relaxivity values for GdDOTA-SiO₂@Au with Au cores of 5 nm are close to those obtained for GdDOTA-SiO₂@QD solution. In addition, a dramatic increase in relaxivity

occurs if 10 nm Au nanoparticles are used instead of 5 nm Au cores. Together, this suggests that the surface area of the nanoparticle rather than the physical nature of the underlying core is the key factor for increased relaxivities.

4. Discussion

We have presented a paramagnetic nanoprobe of about 10-15 nm in diameter that consists in an inner inorganic particle protected with an ultra-thin silica shell to which several chelated paramagnetic ions are covalently linked. Before discussing the properties of such probes as MRI contrast agents and the advantages of using a silica shell in biomedical applications, we first comment on the synthetic route to grow an ultrathin paramagnetic silica shell around nanocrystals of different nature.

Growing a nm-thin silica shell around Au colloids and other cores: The synthesis and use of semiconductor QDs coated with an ultra thin silica shell has been thoroughly described.^{15, 16} In this paper we have extended the procedure to embed Au colloids of 5 nm and 10 nm diameter into a thin silica shell. The synthesis of silica shells around Au cores has been detailed in Liz-Marzan *et al.* pioneering work.²⁷ The authors used a 15 nm Au seed and showed how to grow thick shells (up to > 80 nm) over a period of several days.

Two main issues in growing a silica shell around Au seeds are the avoidance of cross-linking between nanoparticles and the control of the polymerization rate. The latter calls for the use of an anhydrous solvent, while the former calls for diluted solutions of

nanoparticles. This is because polycondensation of methoxysilane into siloxane bonds is driven by hydrolysis and heat/basicity. We want neither of these conditions. First, citrate-stabilized Au colloids are poorly soluble in solvents other than water (including aqueous buffers). Second we should dilute 20 ml of as-purchased 5 nm Au colloids (83 nM) in more than 500 mL of water to start with published protocols. The approach we develop here permits to silanize Au colloids in small volumes (<1-3 ml) at high nanoparticle concentration (> 1 μ M for 5 nm Au). It is amenable to an easy scale-up and is applicable as to the silanization other inorganic cores such as iron oxide ²⁸by imparting them high stability and solubility.

Our silanization protocol for Au colloids calls for an exchange of the citrate capping ligands with a phosphine stabilizer (Bis(*p*-sulfonatophenyl)phenylphosphine), as described thoroughly in the literature. ^{17, 18} Phosphine-stabilized Au colloids are soluble in buffers and water at concentrations ~50-100-fold higher than the original ones; we silanize the nanoparticles at these high concentrations. The phosphine capping is just an intermediate step to allow manipulation of Au colloids in water and preventing their aggregation. To grow the silica shell, phosphine groups are replaced with thiolate primers, specifically mercaptopropyltrimethoxysilane or MPS. Because of the strong affinity between thiols and gold surfaces, the capping exchange is fast (<20 min) and efficient. The methoxysilane or silanol groups of MPS act as an anchor molecule upon which the silica shell forms. The consolidation and polymerization of MPS into a siloxane or silica shell can be controlled by choosing weakly alkaline aqueous solutions (pH~7.5-8) instead of heat. While the shell is slowly forming, fresh MPS and PEG-siloxane are incorporated into the shell. The shell growth is finally quenched by converting the remaining silanol

groups into unreactive methyl groups. At this point silanized Au colloids can be purified from excess silane by dialysis, repeated runs in centricon 100 devices, and size-exclusion column.

The whole procedure for silanizing Au colloids takes about 3 hrs and is performed at particle concentration above $\sim 1 \mu\text{M}$ for 5nm Au cores and above $0.1 \mu\text{M}$ for 10nm cores. We found that the same protocol works for both sizes of Au colloids. There is no evidence of aggregation of particles during the silanization process. The plasmon peaks of citrate-Au solutions and silanized-Au solutions are at the same wavelength ($\sim 524 \text{ nm}$ vs $\sim 526 \text{ nm}$ respectively). The UV-Vis spectrum of silanized Au solutions is stable for weeks, even though silanized Au solutions are stored at high concentrations in a 10 mM phosphate buffer. Gel electrophoresis mobility of silanized Au is qualitatively similar to that of silanized QD nanoparticles.

The ability to grow silica shells around inorganic cores has several advantages: first the nanoparticles are extremely soluble in a wide variety of conditions ($4 < \text{pH} < 11$, and ionic strengths above 1M of phosphate buffer and 50 mM for buffers with divalent ions). Second, the overall size of the nanoparticles remains small since the silica shell only adds a few nm to the particle diameter. We estimate that the silica shell around the 5 nm Au cores is only 2 nm thick. This results in particle size of about 9 nm. Similarly, we estimate that the silica shell adds about 2-4 nm to Au colloids of 10 nm in diameters, with a resulting total size of 15-18 nm. Finally, bioconjugation strategies to attach biomolecules to silica are well-developed. This is illustrated by the covalent linking of GdDOTA to the $\text{SiO}_2@\text{P}$ particles. We link together the thiols of the silica shells with amines groups on the paramagnetic chelated species using the ubiquitous sulfo-SMCC.

The linking protocol follows closely the one we develop to covalently bind DNA to silanized QD.^{29, 30}

Paramagnetic silica shell as a generic scaffold for multivalent contrast agents: Nanoparticles embedded into paramagnetic GdDOTA-SiO₂ shells reach relaxivities that have been matched or surpassed only by highly complex and branched organic dendrimers of generations $N \geq 7$ ³¹ and iron oxide nanoparticles with core size above 20-40 nm.⁶ Our MRI probes represent a compromise between exceedingly high relaxivity values obtained with large iron oxide particles and small “protein-like” sizes of branched dendrimers. In addition, our MRI probes can be made in a few hours, in an Eppendorf tube using water as main solvent and a benchtop centrifuge for purification. The design has considerable potential for scale-up and plenty of room for tailoring the surface to specific biological applications (linking of targeting agent for instance).

This design for this MRI contrast agent combines an outer paramagnetic shell with an inorganic core. It can generally be described as GdDOTA-SiO₂@Particle. The great interest in such design is the possibility to select a material as the core particle that provides a signature orthogonal to the one provided by the paramagnetic GdDOTA-SiO₂ shell. For example, the core provides an optical component (fluorescence), while the chelated paramagnetic ions linked to the outer shell contribute to MRI relaxivity. The strength of the design consists in the fact that the paramagnetic silica shell does not interfere with optical properties of the inorganic cores. For instance, the position of the plasmon peak of GdDOTA-SiO₂@Au shifts by less than 2 nm compared to citrate-stabilized Au. Similarly, the UV-Vis absorption and fluorescence emission of GdDOTA-SiO₂@QD are virtually similar to those of TOPO-capped QD. Although detailed

investigations of fluorescence properties have not been performed in detail, side-to-side comparison of QD and GdDOTA-SiO₂@QD excited with a hand-held UV lamp shows that both solutions have fluorescence properties (color and intensity) undistinguishable to the naked eye.

This approach, where optical and MRI properties arise from physically separated and weakly interacting entities, present several advantages over other approaches devised to make multivalent probes. For instance, attempts to dope fluorescent core/shell ZnS/CdSe nanoparticles with Mn impurities inside the ZnS shell produce multimodal nanoparticles with quenched fluorescence and a relatively weak magnetic contribution.

Achieving high relaxivities does not require the use of an inorganic core of a specific nature, because the MRI contrast power is carried only by the paramagnetic silica shell. In fact, any inorganic core that can be embedded into silica can be used as seed for high relaxivity contrast agents. This includes CdSe/ZnS, CdTe, Au, Ag, and any oxide nanocrystals, including very small superparamagnetic iron oxide nanoparticles²⁸. In fact, it may be even possible to optimize the design and reach an even higher relaxivity values by other combinations of lanthanide ions, chelators and inorganic nanoparticles. Of great interest is the use of a superparamagnetic core in conjunction with the paramagnetic shell to enhance the sensitivity even further.

Mechanisms responsible for relaxivity enhancement: In this study, we found that GdDOTA-SiO₂@QD and GdDOTA-SiO₂@Au with a diameter of about 8-10 nm (5 nm cores + 2 nm silica shell) exhibit relaxivities in excess of $r_1 \sim 1000-2000 \text{ mM}^{-1}\text{s}^{-1}$ and $r_2 \sim 3000 \text{ mM}^{-1}\text{s}^{-1}$ and are detectable at $\sim 100 \text{ nM}$ concentrations. One obvious reason for this enhanced relaxivity is the number of GdDOTA molecules that decorate the silica

surface. Chemical analysis indicates that about 45-50 GdDOTA are covering the silica surface of SiO₂@Au with 5 nm cores. More than 250-300 GdDOTA were measured around SiO₂@Au with 10 nm cores. As a result, relaxivities skyrocketed to ~16'000 mM⁻¹s⁻¹ and the detection limit plunged in the 10 nM range.

While the number of paramagnetic chelated ions is certainly the major factor in enhancing the total relaxivity, more subtle effects may also contribute to it. They manifest themselves by increasing the contribution of every individual GdDOTA to the total relaxivity. To see this, the total relaxivities of the samples is divided by the number of GdDOTA and converted into an ion relaxivity. This translates into T₁ and T₂ ion relaxivities at 60 MHz of ~ 23 mM⁻¹s⁻¹ and 54 mM⁻¹s⁻¹ for GdDOTA-SiO₂@P and only 3-5 mM⁻¹ s⁻¹ for unbound GdDOTA.

Increased ion relaxivities are expected when GdDOTA is constrained in its rotational motion. This is observed for macromolecular conjugates ¹⁰. It has also been observed in a recent study where paramagnetic lipids were desorbed around a QD nanoparticle and relaxivities in the range of 2000 mM⁻¹s⁻¹ at 60 MHz were measured. ³² It was determined that about ~150 GdDOTA-lipids were surrounding the nanoparticle scaffold. Consequently, at 60 MHz, every Gd ion in the lipid payload was contributing by about 12 mM⁻¹s⁻¹ to the spin-lattice relaxivity r₁ and by about 18 mM⁻¹s⁻¹ to the spin-spin relaxivity r₂. It was mainly assumed that the increase in ion relaxivity came from the reduced tumbling rate of GdDOTA due to its covalent linking to a higher molecular weight macromolecule and the consequent increase in its rotational correlation time.

Our probes are quite similar to these, except that the paramagnetic lipid coat is replaced with a thinner paramagnetic silica shell. Yet, this change alone seems to affect

the ion relaxivities. The values for the paramagnetic silica shell are 2 to 3 times higher than the values obtained with the paramagnetic lipid. As a result, our probes reach the same sensitivity with twice to three times less Gd^{3+} load. Although the rotational correlation time may be slightly different for these two systems, it is unlikely that it alone accounts for this large difference in relaxivity. We believe that a second reason for the increase in ion relaxivity comes from the very hydrophilic environment around GdDOTA provided by the silica shell. Silica is much more hydrophilic than lipids. It is likely that it generates a denser water solvation shell around the nanoparticle which forces more protons to interact with GdDOTA. We rule out that the high permanent electric dipole of the $\text{SiO}_2@\text{QD}$ can affect the dynamic of water proton markedly, because the replacement of a QD core with a dipole-free Au particle of similar size produces a similar increase in ion relaxivity.

It is instructive to observe that ion relaxivities for $\text{GdDOTA-SiO}_2@\text{P}$ are very close to those obtained for high generation organic dendrimer-GdDOTA ($N>7$) where ion relaxivities reach a plateau at $35 \text{ mM}^{-1}\text{s}^{-1}$ and $43 \text{ mM}^{-1}\text{s}^{-1}$ respectively.³¹ Like our paramagnetic probes, these dendrimers are efficient T_2 contrast agent while the GdDOTA moieties by themselves are only T_1 contrast agents. Data for $\text{GdDOTA-SiO}_2@\text{P}$ suggest that their MRI properties can be satisfactorily described within the framework of the classical relaxation theory.¹⁰

Relaxivity of $\text{GdDOTA-SiO}_2@\text{nanoparticles}$ vs other contrast agents: The probes embedded into the paramagnetic GdDOTA-SiO_2 shell reach relaxivities of a few thousands $\text{mM}^{-1}\text{s}^{-1}$ well in excess of the few, to a few tens $\text{mM}^{-1}\text{s}^{-1}$ observed for

individual GdDOTA.¹⁰ They compared favorably even with the most promising new types of MRI contrast agent technology based on ultrasmall iron oxide nanoparticles.³³⁻³⁵ Recent reports indicated that Au-coated iron oxide nanoparticles with a size of 19 nm have ion relaxivities of only 3 mM⁻¹s⁻¹ in the 30-50 MHz range.³⁵ Considering that there are a few thousand Fe ions per nanoparticle, this translates into total relaxivities estimated in the ~10000-20000 mM⁻¹s⁻¹ range. At this size range however, the surface chemistry of iron oxide is not yet well developed. Nanoparticles are often solubilized by ligand exchange,^{33, 34} although such approach is unlikely to have widespread use *in vivo* because of the non-covalent nature of the passivating bonds. Cross-linked, stable and robust shells are necessary. This is why intense efforts are underway to embed iron oxide particles into biocompatible and robust coatings. Silica shells²⁸, Au shells³⁵ and clustering into polymeric micelles¹⁴ have been investigated. However, extensive aggregation is often observed for several of these formulations. Poor solubility does not influence too much the MRI properties of the solutions, but hinders the use of such formulations for *in vivo* applications. Surface chemistry is better controlled in commercial contrast agents made of larger iron oxide nanoparticle cores (>50 nm) embedded in a protective polymer shell (usually dextran). The polymer shell, that protects the inorganic core from aqueous environment, adds several tens of nanometers to the size of the particles. As a result, water protons are always kept at a distance exceeding a few tens of nanometers from the iron oxide core. Since the interaction of the (superpara)-magnetic core with water protons operates through a magnetic dipole coupling, it scales as 1/d³ (where d is the distance between the magnetic center and the water protons) and vanishes quickly as the protective coating gets large. Key to a strong interaction consists in bringing protons

closer to the contrast agent by reducing the distance between the proton and the magnetic contrast agent. This is what our approach achieves. The paramagnetic molecules providing contrast power for MRI are decorating the outside surface of the silica shell and therefore are in direct contact with water protons.

Advantages of a paramagnetic silica shell coating in biology: Bare nanocrystals tend to aggregate in aqueous solutions and adsorb plasma or other proteins through non-specific interactions. To prevent their aggregation and tailor their surface properties, nanocrystals must be stabilized and embedded into a biocompatible and robust shell. Silica presents several advantages over polymer-based shells. Unlike polymers, silica is not subjected to microbial or macrophage attack and it neither swells nor changes shape and porosity with changing pHs. Silica is chemically inert and therefore does not influence the redox reaction of the core surface. Furthermore, the chemistry to functionalize silica is well-developed. It is almost straightforward to introduce thiols, amine or carboxylic groups onto a silica surface. The groups can be further derivatized with targeting biomolecules using established bioconjugation techniques.²¹ Finally, it is much easier to control the polymerization of siloxane into silica (and hence the size of the silica shell) than it is to control the thickness of a polymer-based coating. For example, FCS and dynamic light scattering measurements indicated that while silica coated Qdot have a hydrodynamic radius of 9-11 nm, polymer-embedded Qdots have a radius close to 30 nm³⁶ and Au-coated iron oxide close to ~250 nm.³⁵

Silica has other advantages over polymeric nanoparticles that have emerged in recent studies. First, the silica-coated nanoparticles exhibit much smaller cytotoxicity than polymer-coated nanoparticles.³⁷ Even more remarkable, silica-coated nanoparticles

were shown to have negligible perturbation on the gene expression patterns of lung and skin epithelial cells.³⁸ This suggests that silica-coated nanoparticles have a minimal interference with the normal physiology of these cell lines. In other words, the presence of these local probes inside the cell does not affect the expression pattern of critical genes and therefore does not influence significantly most cell functions. Toxicity studies at the gene expression level of silica-coated nanoparticles on other cell lines, tissues or animal models has not been investigated so far and is undoubtedly an emerging research area. In addition, silica-coated nanoparticles can be functionalized with a wide array of targeting biomolecules.^{16, 29, 30} They can be biologically programmed to recognize critical phosphorylation sites, nucleases, motor proteins, or surface receptors on organelles such as the cell nucleus.³⁹ Silica-coated nanoparticles may play a key role for cell biology for deciphering molecular pathways.

For *in vivo* molecular imaging and drug delivery, the size of the probes is a key parameter. It is likely that if the probes are too big, they have a reduced ability to diffuse in tumoral microvasculature and transverse the extracellular matrix, as they have a limited ability to cross cellular membranes. Although the ideal size of a probe is not known, we hypothesize that probes with sizes in the 10-20 nm range, as the ones we developed, represent the best candidates since they mimic the size of therapeutic antibodies.

Biological applications resulting from high relaxivities: With this remarkable relaxivity enhancement and sensitivity, silica-coated nanoparticles have a large number of potential applications in MRI. These include cell therapies, include stem cell tracking, where a small number of parent cells can be labeled, implanted and their progeny

followed *in vivo*.³³ Another application is guided surgery where biologically programmed particles can target infected organs and reveal their precise location. Common to all these biomedical applications is the ability to use nanoprobe *in vivo*. To date, most inorganic nanoparticles perform poorly in *in vitro* assays. Nanoparticles often aggregate under changing pH conditions and ionic buffer strengths. Although specific labeling of membrane proteins has been widely reported, intracellular targeting remains a challenging task. There are no established protocols to transfect live cells without external intervention like microinjection. Finally, toxicology effects have been barely investigated in cell cultures, let alone in *in vivo* studies.

Yet, the use of silica-coated nanoparticles in biomedicine has shown some promising results. Silica coated QD have extended solubility to the point where aggregation is no longer an issue, they can be conjugated to targeting biomolecules and in some specific – yet still not fully understood – cases, the particles are able to spontaneously transfect live cells. Finally, toxicology studies, done at the gene expression level, reveal the lack of adverse effect of silica-coated QD on a few cell lines. We feel that silica-coated nanoparticles present a starting point where to investigate the possibility to apply nanotechnology to medicine.

4. Conclusions

We have described a strategy to embed inorganic nanoprobe into a paramagnetic silica shell. The shell is rendered paramagnetic by covalently linking GdDOTA to its

surface. Once attached to the surface, each of these contrast agent units exhibit T_1 and T_2 ion relaxivities at clinical fields that are respectively 9 and 18 times larger than the ion relaxivities of unbound GdDOTA. We provide evidence that the increase is not related to the nature of the inorganic core but most likely to the fact that GdDOTA are bound to a hydrophilic silica surface which reduces their rotational motion.

What matters for imaging purposes is not primarily the ion relaxivity of a contrast agent, but its total relaxivity. Similar to the case of dendrimeric polymers, multiple GdDOTA can be anchored onto the surface of a single silica nanoparticle. Since the ion relaxivity is additive, we have measured T_1 and T_2 relaxivities at room temperatures and at clinical fields in the order of $1000\text{--}3000\text{ mM}^{-1}\text{ s}^{-1}$ for GdDOTA-SiO₂@Particle with particle cores of 5 nm, resulting from the contribution of ~ 50 GdDOTA. If the particle cores are 10 nm, the surface area of silica shells permits the linking of $\sim 250\text{--}300$ GdDOTA. Remarkably, these latter probes exhibit relaxivities in excess of $15000\text{ mM}^{-1}\text{ s}^{-1}$ at room temperature and clinical fields.

The paramagnetic silica shell has been grown around semiconductor and metallic nanoparticles. There are however no restriction in the use of the core material. It may be envisioned to grow a GdDOTA-SiO₂ around a (superpara)magnetic core such as small SPIO Fe₃O₄ or Fe₂O₃. In that configuration, perturbation in the dynamic response of water protons will come from the presence of the paramagnetic GdDOTA-SiO₂ shell and from the inner SPIO cores. We expect such systems to present even higher relaxivities values than the ones obtained here.

In addition, it is straightforward to modify the design of the construct into a PET probe. For that, only a handful of DOTA have to be loaded with a radioisotope (i.e. ⁶⁴Cu)

instead of Gd^{3+} . The overall probe will maintain a unique surface chemistry. However since PET sensitivity far exceeds MRI sensitivity, it is possible to test basic properties of the probes, such as their potential leakage from the vascular system, their non-specific uptake by different organs, or their ability to recognize tumors. These studies, currently underway, will provide powerful information on the compatibility and utility of inorganic nanoprobes for in vivo imaging.

Acknowledgements

We would like to thank Giulia Galli, Natalia Zaitseva for their support. This work was performed under the auspices of the U.S. Dept. of Energy at the University of California/Lawrence Livermore National Laboratory under contract no. W-7405-Eng-48.

Figure Captions

Scheme 1: Scheme for the preparation of the paramagnetic probes. Gadolinium chloride is reacted with a DOTA complex under controlled acidic conditions at 80°C.

Subsequently, the amine group is converted into a maleimide group which will react with thiols on the silica-coated nanoparticles. The payload of GdDOTA per nanoparticles depends on the surface area, varying around 50 up to several hundreds.

Figure 1 The spin-lattice relaxation MRI images (T_1 weighted images) taken using a Fast Imaging with Steady State Precession (FISP) with inversion recovery (IR) sequence of PBS buffer, $\text{SiO}_2\text{@QD}$, DOTA- $\text{SiO}_2\text{@QD}$ lacking the paramagnetic load and GdDOTA- $\text{SiO}_2\text{@QD}$, at 400MHz and room temperature. Only the paramagnetic silica-coated nanoparticles exhibit a contrast.

Figure 2: A spin-spin relaxation MRI images (T_2 weighted images) were taken using a Multi Slice Multi Echo (MSME) sequence of PBS buffer, $\text{SiO}_2\text{@QD}$, DOTA- $\text{SiO}_2\text{@QD}$ lacking the paramagnetic load and GdDOTA- $\text{SiO}_2\text{@QD}$, at 400MHz and room temperature.

Figure 3: a) T_1 and T_2 MRI maps taken at various concentrations of GdDOTA- $\text{SiO}_2\text{@QD}$ ranging from 0.125 μM to 4 μM at 400 MHz and at room temperature. During the same experiment, Gd-DOTA at a Gd^{3+} concentration of 390 μM and a phosphate buffer have also been imaged as a reference controls. **b)** Plots of $1/T_1$ and $1/T_2$ verses nanoparticle concentration are shown for the same samples. The slopes represent

the relaxivity and correspond to $r_1=807.9 \pm 15.4 \text{ mM}^{-1}\text{s}^{-1}$ and $r_2=3003.5 \pm 57.1 \text{ mM}^{-1}\text{s}^{-1}$.

Ion relaxivities, i.e. relaxivity per Gd ion, are $r_1=17.9 \pm 0.34 \text{ mM}^{-1}\text{s}^{-1}$ and $r_2=67 \pm 1 \text{ mM}^{-1}\text{s}^{-1}$.

Figure 4. Relaxivities of GdDOTA-SiO₂@QD as a function of the proton Larmor frequency. The lines are guides for the eyes. The trend is similar to that of unbound GdDOTA. Notice however how the T₂ relaxivity is always larger than the longitudinal one.

Table of Tables

Table 1: T₁ and T₂ values of PBS buffer, SiO₂@QD, DOTA-SiO₂@QD lacking the paramagnetic load and GdDOTA-SiO₂@QD, and various concentrations of GdDOTA from 0.39mM to 7.35mM at 400MHz and at room temperature.

Table 2: Relaxivity values at different fields measured at room temperature. In parenthesis is the value of the Gd³⁺ ion relaxivity. This is deduced from the measurement of Gd concentration per nanoparticles with ICP-MS. The bottom line serves as comparison with the Gd³⁺ ion relaxivity of unbound GdDOTA.

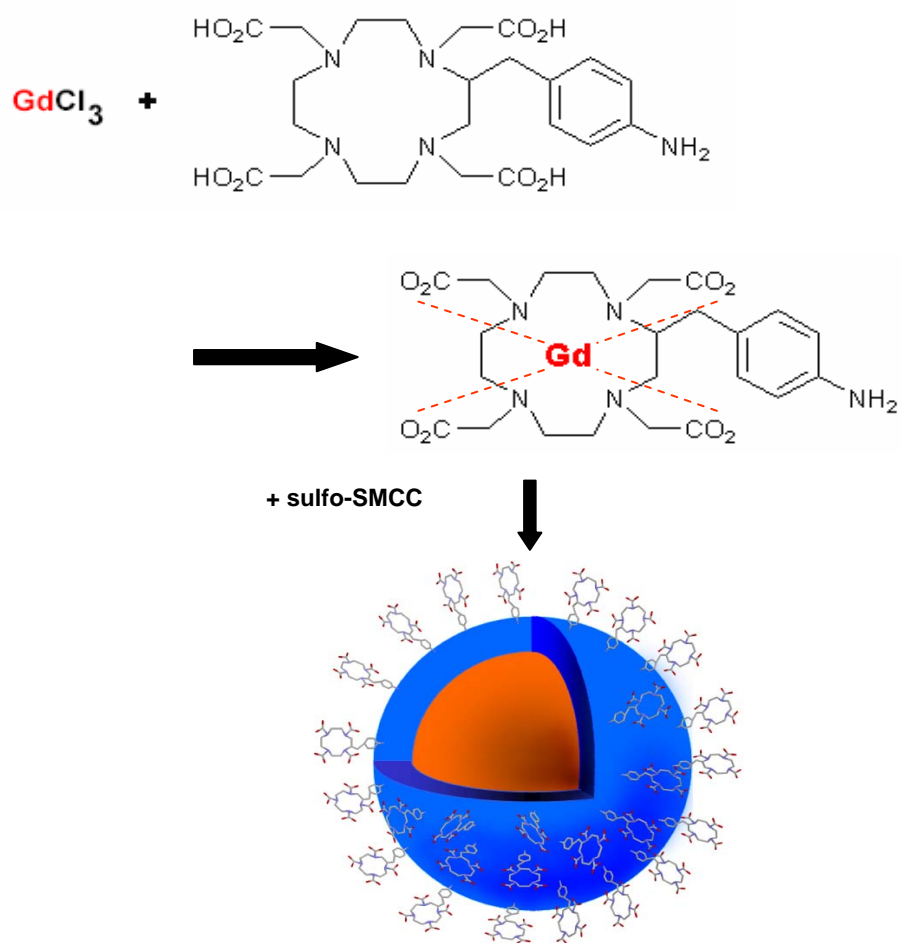
References

1. Tsien, R. Y., Imaging imaging's future. *Nature Cell Biology* 2003, SS16-SS21.
2. Alivisatos, A. P.; Gu, W.; Larabell, C., Quantum Dots as Cellular Probes. *Annu Rev Biomed Eng* 2004.
3. Dahan, M., Watching the dynamics of individual proteins in live cells using quantum dots. *Journal of Histochemistry & Cytochemistry* 2004, 52, S18-S18.
4. Courty, S.; Luccardini, C.; Bellaiche, Y.; Cappello, G.; Dahan, M., Tracking Individual Kinesin Motors in Living Cells Using Single Quantum-Dot Imaging. *Nano Lett.* 2006, 6, (7), 1491 -1495.
5. Bremer, C.; Weissleder, R., Molecular imaging - In vivo imaging of gene expression: MR and optical technologies. *Academic Radiology* 2001, 8, (1), 15-23.
6. Gimi, B.; Pathak, A. P.; Ackerstaff, E.; Glunde, K. A., D.; Bhujwala, Z. M., Molecular imaging of cancer: applications of magnetic resonance methods. *Proceedings of the IEEE* 2005, 93, (4), 784-799.
7. Weissleder, R., A clearer vision for in vivo imaging. *Nature Biotechnology* 2001, 19, (4), 316-317.
8. Weissleder, R., Scaling down imaging: Molecular mapping of cancer in mice. *Nature Reviews Cancer* 2002, 2, (1), 11-18.
9. Allport, J. R.; Weissleder, R., In vivo imaging of gene and cell therapies. *Experimental Hematology* 2001, 29, (11), 1237-1246.
10. Caravan, P.; Ellison, J. J.; McMurry, T. J.; Laufer, R. B., Gadolinium(III) chelates as MRI contrast agents: Structure, dynamics, and applications. *Chemical Reviews* 1999, 99, (9), 2293-2352.
11. Jacques, V.; Desreux, J. F., New Classes of MRI Contrast Agents. *Topics in Current Chemistry* 2002, 221, 123-164.
12. Hajela, S.; Botta, M.; Giraudo, S.; Xu, J.; Raymond, K. N.; Aime, S., A Tris-hydroxymethyl-Substituted Derivative of Gd-TREN-Me-3,2-HOPO: An MRI Relaxation Agent with Improved Efficiency. *J. Am. Chem. Soc.* 2000, 122, 11228-11229.
13. Thompson, M. K.; Botta, M.; Nicolle, G.; Helm, L.; Aime, S.; Merbach, A. E.; Raymond, K. N., A highly stable Gadolinium complex with fast, associative mechanism of water exchange. *J. Am. Chem. Soc.* 2003, 125, 14274-14275.
14. H. Ai; Flask, C.; Weinberg, B., X. T. S. M.; Pagel, D.; Farrell, D.; Duerk, J.; Gao, J., Magnetite-Loaded Polymeric Micelles as Ultrasensitive Magnetic-Resonance Probes. *Advanced Materials* 2005, 17, (16), 1949-1952.

15. Gerion, D.; Pinaud, F.; Williams, S. C.; Parak, W. J.; Zanchet, D.; Weiss, S.; Alivisatos, A. P., Synthesis and properties of biocompatible water-soluble silica-coated CdSe/ZnS semiconductor quantum dots. *Journal of Physical Chemistry B* 2001, 105, (37), 8861-8871.
16. Wolcott, A.; Gerion, D.; Visconte, M.; Sun, J.; Schwartzberg, A.; Chen, S.; Zhang, J. Z., Silica-coated CdTe quantum dots functionalized with thiols for bioconjugation to IgG Proteins. *J. Phys. Chem. B* 2006, 110, 5779-5789.
17. Zanchet, D.; Micheel, C. M.; Parak, W. J.; Gerion, D.; Alivisatos, A. P., Electrophoretic isolation of discrete Au nanocrystal/DNA conjugates. *Nano Lett.* 2001, 1, 32-35.
18. Loweth, C. J.; Caldwell, W. B.; Peng, X.; Alivisatos, A. P.; Schultz, P. G., DNA-Based Assembly of Gold Nanocrystals. *Angewandte Chemie International Edition* 1999, 38, (12), 1808-1812.
19. Wang, X. Y.; Jin, T. Z.; Comblin, V.; Lopezmut, A.; Merciny, E.; Desreux, J. F., A Kinetic Investigation of the Lanthanide DOTA Chelates - Stability and Rates of Formation and of Dissociation of a Macrocyclic Gadolinium(III) Polyaza Polycarboxylic MRI Contrast Agent. *Inorganic Chemistry* 1992, 31, (6), 1095-1099.
20. Prantner, A. M.; Sharma, V.; Garbow, J. R.; Piwnica-Worms, D., Synthesis and characterization of a Gd-DOTA-D-permeation peptide for magnetic resonance relaxation enhancement of intracellular targets. *Molecular Imaging* 2003, 2, (4), 333-341.
21. Hermanson, G. T., *Bioconjugate techniques*. Academic Press, Inc.: San Diego, CA, 1996.
22. Yu, W. W.; Qu, L. H.; Guo, W. Z.; Peng, X. G., Experimental determination of the extinction coefficient of CdTe, CdSe, and CdS nanocrystals. *Chemistry of Materials* 2003, 15, (14), 2854-2860.
23. Lauffer, R. B., Paramagnetic Metal-Complexes as Water Proton Relaxation Agents for Nmr Imaging - Theory and Design. *Chemical Reviews* 1987, 87, (5), 901-927.
24. Aime, S.; Botta, M.; Fasano, M.; Geninatti Crich, S.; Terreno, E., ¹H and ¹⁷O-NMR relaxometric investigations of paramagnetic contrast agents for MRI. Clues for higher relaxivities. *Coordination Chemistry Reviews* 1999, 185-186, 321-333.
25. Aime, S.; Cabella, C.; Colombatto, S.; Crich, S. G.; Gianolio, E.; Maggioni, F., Insights into the use of paramagnetic Gd(III) complexes in MR-molecular imaging investigations. *Journal of Magnetic Resonance Imaging* 2002, 16, (4), 394-406.
26. Li, L.-s.; Alivisatos, A. P., Origin and Scaling of the Permanent Dipole Moment in CdSe Nanorods. *Physical Review Letters* 2003, 90, (9), 097402.
27. Liz-Marzan, L. M.; Giersig, M.; Mulvaney, P., Synthesis of Nanosized Gold-Silica Core-Shell Particles. *Langmuir* 1996, 12, (18), 4329-4335.

28. He, Y. P.; Wang, S. Q.; Li, C. R.; Miao, Y. M.; Wu, Z. Y.; Zou, B. S., Synthesis and characterization of functionalized silica-coated Fe₃O₄ superparamagnetic nanocrystals for biological applications. *J. Phys. D: Appl. Phys.* 2005, 38, 1342-1350.
29. Gerion, D.; Parak, W. J.; Williams, S. C.; Zanchet, D.; Micheel, C. M.; Alivisatos, A. P., Sorting fluorescent nanocrystals with DNA. *Journal of the American Chemical Society* 2002, 124, (24), 7070-7074.
30. Gerion, D.; Parak, W. J.; Zanchet, D.; Woerz, A. S.; Pellegrino, T.; Micheel, C. M.; Williams, S. C.; Seitz, M.; Bruehl, R. E.; Bryant, Z.; Bustamante, C.; Bertozzi, C. R.; Alivisatos, A. P., Conjugation of DNA to silanized colloidal semiconductor nanocrystalline quantum dots. *Chemistry of Materials* 2002, 14, (5), 2113-2119.
31. Bryant Jr, L. H.; Brechbiel, M. W.; Wu, C.; Bulte, J. W. M.; Herynek, V.; Frank, J. A., Synthesis and relaxometry of high-generation (G = 5, 7, 9, and 10) PAMAM dendrimer-DOTA-gadolinium chelates. *Journal of Magnetic Resonance Imaging* 1999, 9, (2), 348-352.
32. Mulder, W. J. M.; Koole, R.; Brandwijk, R. J.; Storm, G.; Chin, P. T. K.; Strijkers, G. J.; deMelloDonega, C.; Nicolay, K.; Griffioen, A. W., Quantum Dots with a Paramagnetic Coating as a Bimodal Molecular Imaging Probe. *Nano Lett.* 2006, 6, (1), 1-6.
33. Song, H.-T.; Choi, J.-s.; Huh, Y.-M.; Kim, S.; Jun, Y.-w.; Suh, J.-S.; Cheon, J., Surface Modulation of Magnetic Nanocrystals in the Development of Highly Efficient Magnetic Resonance Probes for Intracellular Labeling. *J. Am. Chem. Soc.*, 2005, 127, (28), 9992 -9993.
34. Jun, Y.-w.; Huh, Y.-M.; Choi, J.-s.; Lee, J.-H.; Song, H.-T.; Kim, S.; Yoon, S.; Kim, K.-S.; Shin, J.-S.; Suh, J.-S.; Cheon, J., Nanoscale Size Effect of Magnetic Nanocrystals and Their Utilization for Cancer Diagnosis via Magnetic Resonance Imaging. *J. Am. Chem. Soc.* 2005, 127, (16), 5732 -5733.
35. Cho, S.-J.; Jarrett, B. R.; Louie, A. Y.; Kauzlarich, S. M., Gold-coated iron nanoparticles: a novel magnetic resonance agent for T1 and T2 weighted imaging. *Nanotechnology* 2006, 17, 640-644.
36. Doose, S.; Tsay, J. M.; Pinaud, F.; Weiss, S., Comparison of Photophysical and Colloidal Properties of Biocompatible Semiconductor Nanocrystals Using Fluorescence Correlation Spectroscopy. *Anal. Chem.* 2005, 77, (7), 2235 -2242.
37. Kirchner, C.; Liedl, T.; Kudera, S.; Pellegrino, T.; Muñoz Javier, A.; Gaub, H. E.; Stölzle, S.; Fertig, N.; Parak, W. J., Cytotoxicity of Colloidal CdSe and CdSe/ZnS Nanoparticles. *Nano Letters* 2005, 5, (2), 331-338.
38. Zhang, T.; Gerion, D.; Stilwell, J.; Ding, L.; Elboudwarej, O.; Cooke, P. A.; Alivisatos, A. P.; Gray, J. W.; Chen, F., Cellular Effect of high doses of silica-coated Qdots profiled with high-throughput gene expression analysis and high content cellomics measurements. *Nano Lett.* 2006, 6, (4), 800-808.

39. Chen, F.; Gerion, D., Fluorescent CdSe/ZnS nanocrystal-peptide conjugates for long-term, nontoxic imaging and nuclear targeting in living cells. *Nano Letters* 2004, 4, (10), 1827-1832.



Scheme 1

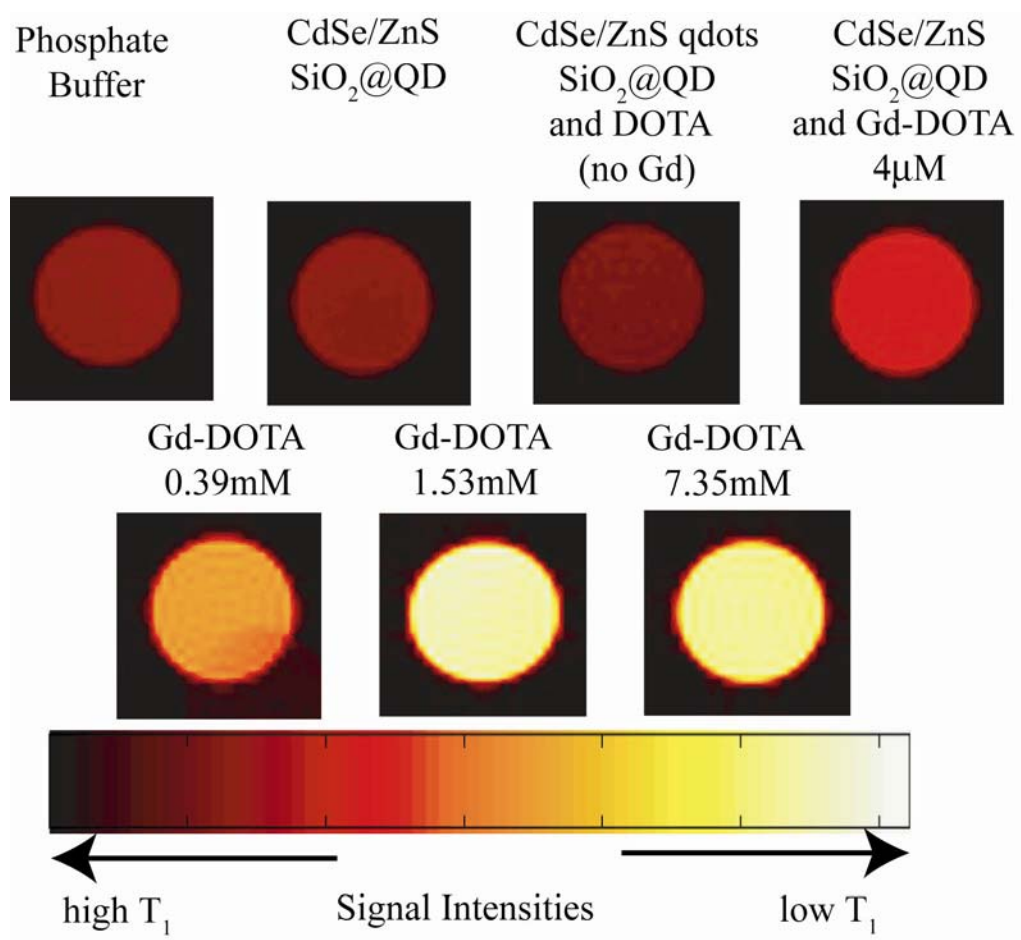


Figure 1

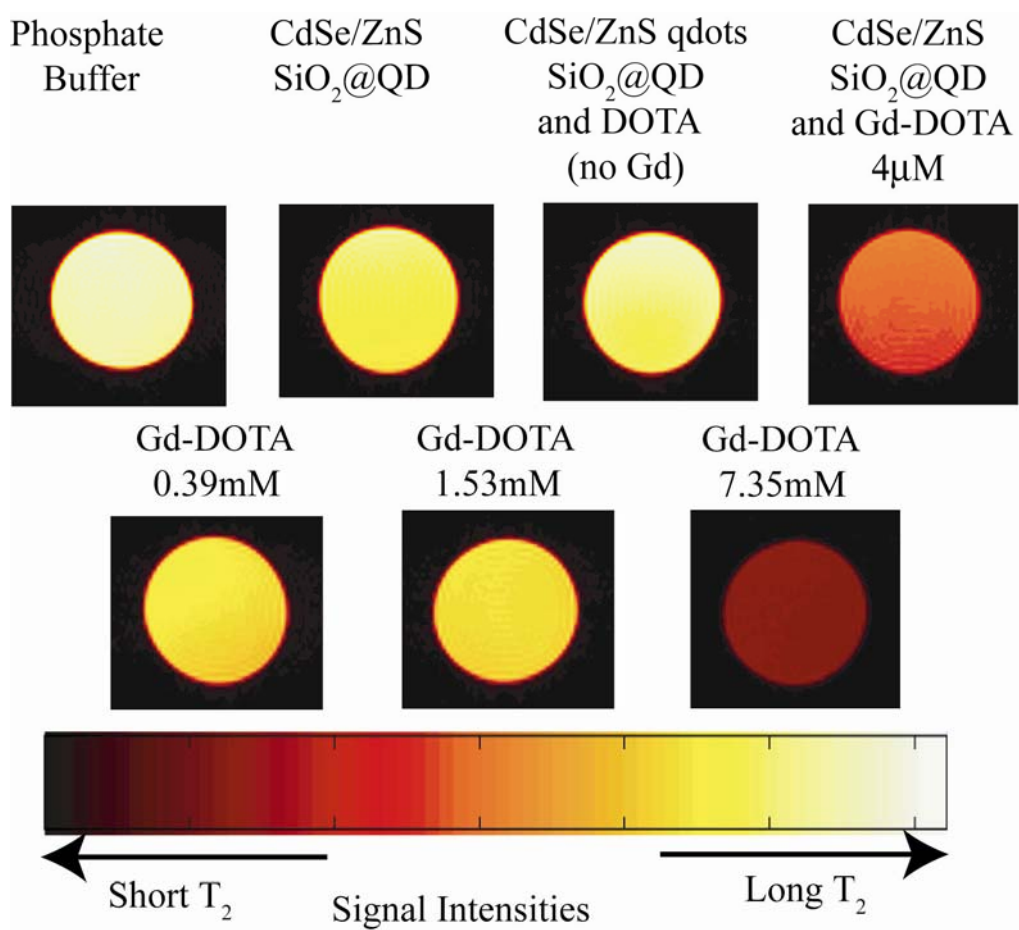


Figure 2

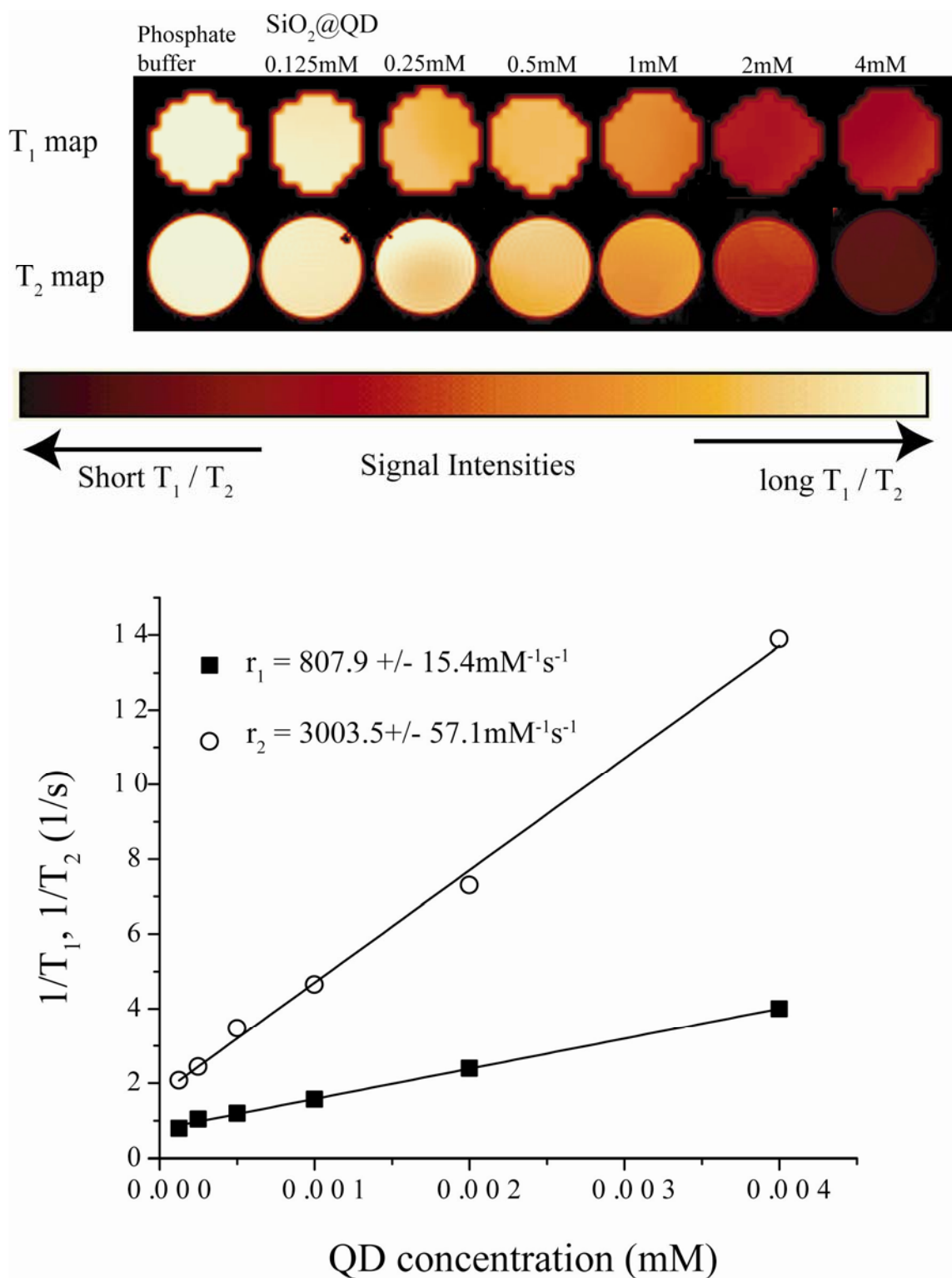


Figure 3

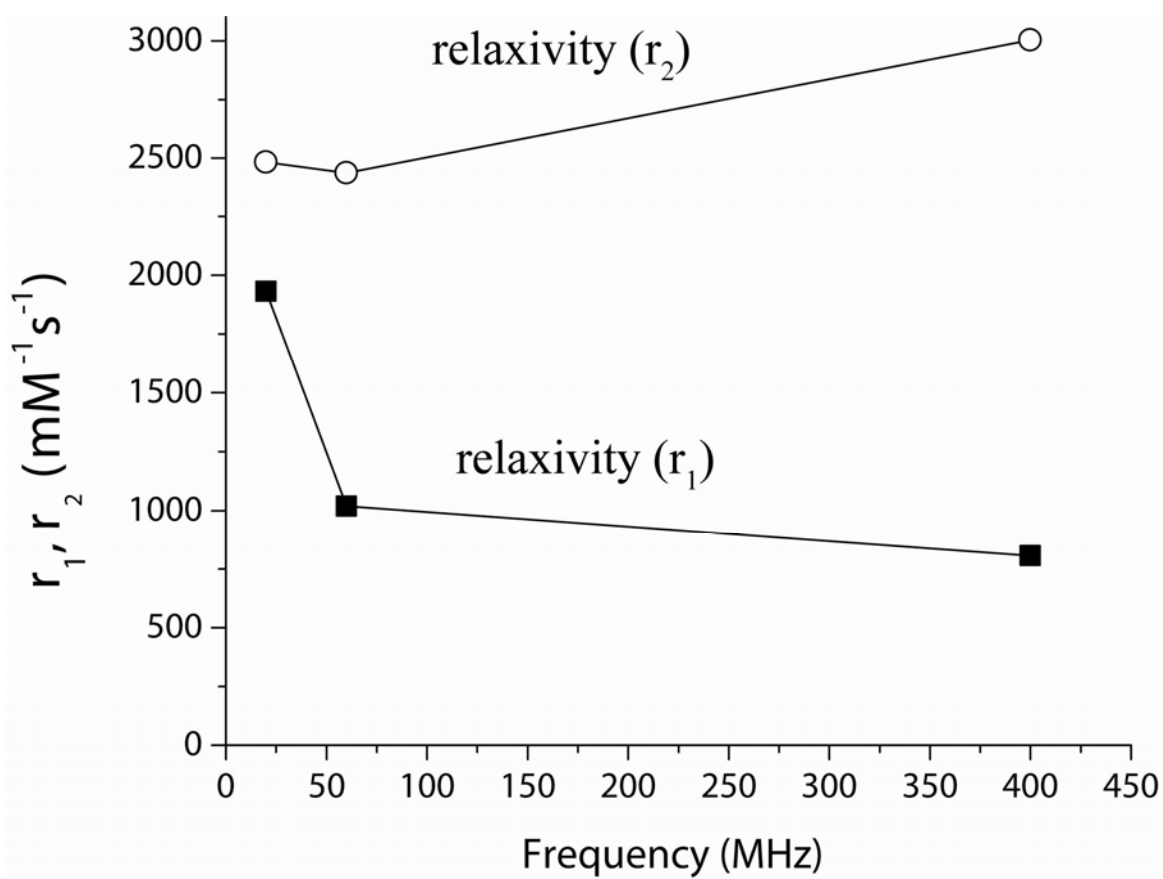


Figure 4

Sample name	T1 [ms]	T2 [ms]
PBS buffer	414	425
SiO ₂ @QD	600	345
DOTA-SiO ₂ @QD	577	347
GdDOTA-SiO ₂ @QD	186	77
GdDOTA, 390 mM	237	201
GdDOTA, 1530 mM	147	123
GdDOTA, 7350 mM	59	37

Table 1

Frequency [MHz]	R ₁ relaxivity [mM ⁻¹ s ⁻¹]	R ₂ relaxivity [mM ⁻¹ s ⁻¹]
20	1932.0 ± 36.7 (42.9 ± 0.8)	2483.6 ± 47.2 (55.2 ± 1.1)
60	1018.6 ± 19.4 (22.6 ± 0.4)	2438.1 ± 46.3 (54.2 ± 1.0)
400	807.9 ± 15.4 (17.9 ± 0.3)	3003.5 ± 51.7 (66.7 ± 1.3)
GdDOTA 20-100	3-5	3-5

Table 2